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The Effects of Hyperthermia and Hyperthermia Plus Microwaves on Rat Brain Energy Metabolism

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The effects of hyperthermia, alone and in conjunction with microwave exposure, on brain energetics were studied in anesthetized male Sprague-Dawley rats. The effect of temperature on adenosine triphosphate concentration [ATP] and creatine phosphate concentration [CP] was determined in the brains of rats that were maintained at 35.6, 37.0, 39.0, and 41.0 °C. At 37, 39, and 41 °C brain [ATP] and [CP] were down 6.0, 10.8, and 29.2%, and 19.6, 28.7, and 44%, respectively, from the 35.6 °C control concentrations. Exposure of the brain to 591-MHz radiation at 13.8 mW/cm² for 0.5, 1.0, 3.0, and 5.0 min caused further decreases (below those observed for 30 °C hyperthermia only) of 16.0, 29.8, 22.5, and 12.3% in brain [ATP], and of 15.6, 25.1, 21.4, and 25.9% in brain [CP] after 0.5, 1.0, 3.0, and 5.0 min, respectively. Recording of brain reduced nicotinamide adenine dinucleotide (NADH) fluorescence before, during, and after microwave exposure showed an increase in NADH fluorescence during microwave exposure that returned to preexposure levels within 1 min postexposure. Continuous recording of brain temperatures during microwave exposures showed that brain temperature varied between -0.1 and +0.05 °C. Since the microwave exposures did not induce tissue hyperthermia, it is concluded that direct microwave interaction at the subcellular level is responsible for the observed decrease in [ATP] and [CP].

Key words: hyperthermia, microwaves, brain, ATP, CP, energy metabolism

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INTRODUCTION

The question of possible biological effects from microwave exposure that are independent of general tissue hyperthermia has been a matter of controversy for some time [Gavalas et al, 1970; Baranski, 1971; Czerski et al, 1974; Adey, 1975; Bawin et al, 1975; Blackman et al, 1980]. Data from earlier work in our laboratory were consistent with the hypothesis that microwave exposures in vivo inhibit the mitochondrial electron transport chain resulting in decreased adenosine triphosphate concentration [ATP] and creatine phosphate [CP] concentration [Sanders et al (1980)]. This study showed that microwaves induced an increase in reduced nicotinamide adenine dinucleotide (NADH) fluorescence in rat brain with a concomitant decrease in [ATP] and [CP] in the brain of rats exposed to 591 MHz, continuous wave (CW) radiation at 13.8 mW/cm². No significant change in brain temperature was observed even though significant changes were observed in brain [ATP], [CP], and NADH fluorescence.

This study reports the results of experiments performed to determine the effects of hyperthermia alone and in combination with microwaves on energy metabolism as reflected in brain [ATP] and [CP].

METHODS

Animal Preparation and Instrumentation

Male Sprague-Dawley rats (175–225 g), were anesthetized with urethane (1,250–1,350 mg/kg, IP injection). Urethane anesthesia was selected to facilitate sustaining deep anesthesia over a prolonged time interval, and to avoid barbiturate effects on brain energy metabolism [Siesjo, 1978]. A 4×8-mm aperture was made through the skull with a small bone saw after the scalp and muscle were removed above the area to be studied. The sagittal sinus was avoided. The dura remained intact before, during, and after microwave exposures until the time for biochemical assays for [ATP] and [CP].

Animals receiving sham or microwave exposures were positioned under a titania-loaded horn antenna so that only the head and neck were exposed to the microwaves, as previously described [Sanders et al, 1980]. The head of the rat was held rigid in a Teflon head holder for all NADH fluorescence studies. The excitation light (366 nm) of the fluorimeter¹ was focused to a 1- to 2-mm spot on the cerebral cortex. The end of a fiber optic bundle was positioned at approximately 45° with respect to the excitation light beam, and at a distance of 1–2 cm from the excitation focal spot. The fiber optic bundle bifurcates 15 cm from the distal end. Each end is attached to an interference-filter-photomultiplier-op amp-assembly that is connected to a strip chart recorder. One of the two channels has a 460 ± 5 nm half-bandwidth (HBW) interference filter for measuring NADH fluorescence, and the other has a 549 ± 2 nm HBW filter for measuring general brain fluorescence. The latter allowed correction of NADH fluorescence data for the effects of changes in hemoglobin content in the field of study, as detailed by Kramer and Pearlstein [1979]. This proved to be unnecessary in all experiments. All animals appeared to maintain normal

¹The fluorimeter was obtained from Dr. Britton Chance, The Johnson Research Foundation, University of Pennsylvania, Philadelphia, PA 19104.

respiration throughout microwave exposures. No changes were observed in the general brain fluorescence at 549 ± 2 nm during and after the microwave exposures. These results support the observation that normal respiration rate was maintained in animals during microwave exposures. (A decrease in O_2 delivery sufficient to decrease energy metabolism in the brain would cause vascular dilatation and increased hemoglobin in the field resulting in changes in the general brain fluorescence signal at 549 ± 2 nm, which was not observed.)

Microwave Instrumentation and Dosimetry

The output of a microwave generator (AIL 125A) was connected via a circulator (Western Microwaves Model 3JA 2087) and a dual-directional coupler (NARDA Model 3020A) with two HP 435A power meters and detectors (for measuring forward and reflected power) to a dielectric-loaded (titania) horn antenna. The design and construction of this antenna have been described previously [Sanders et al, 1980]. The 3×3 -cm aperture antenna was positioned approximately 3 cm above the surface of the brain and the electric field parallel to the body axis as shown in Figure 1. With the 3-cm spacing, the electric and magnetic field intensities were approximately constant on a plane perpendicular to the direction of energy propagation, ie, the field is approximately a uniform plane wave at the tissue surface. Measurements made with a microwave power density meter (General Microwave Corp. RAHAM Model 2) showed that the radiation pattern from the horn antenna was such that the head of the animal was uniformly irradiated, and most of the torso received power densities below 0.1 mW/cm^2 . The nose and the neck of the animal were not uniformly irradiated.

A prolate spheroid with electric field parallel to the major axis is a reasonable simulation of the volume of the rat's head under these exposure conditions, since the

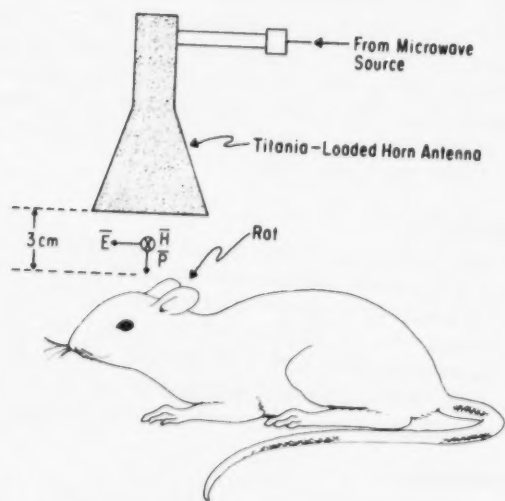


Fig. 1. Horn antenna exposure arrangement. E is the vector electric field intensity. H is the vector magnetic field intensity, and P is the Poynting vector of instantaneous power density.

nose and neck of the animals are partly within the irradiation field of the antenna. This means that the total irradiated volume is not precisely known. To establish upper and lower limits of the specific absorption rate (SAR), we used data at 591 MHz from curves in "The Radiofrequency Radiation Dosimetry Handbook" [Durney et al, 1978]. A 110-cm³ prolate spheroid (semiaxes $a=7$ cm and $b=1.94$ cm) and one of 25 cm³ ($a=3.8$ cm and $b=1.25$ cm) were selected as limiting cases for which the SARs are 0.42 and 0.18 mW/g per mW/cm² respectively. Average values of brain SAR are comparable to, but less than, localized values of SAR that can be estimated from measurements of change in localized temperature per unit time.

The density of absorbed power at a site within the rat brain may be estimated from:

$$P_a = Kc\Delta T/t,$$

where P_a = absorbed power

$$K = 4.186 \text{ J/cal}$$

$$c = \text{specific heat of tissue (g/}^\circ\text{C)}$$

$$\Delta T = \text{temperature change (}^\circ\text{C)}$$

$$t = \text{time(s)}$$

Temperature was measured continuously at a point approximately 2 mm below the surface of the brain and in the middle of the 4×8-mm skull aperture via a 22-gauge thermistor probe (Yellow Springs Instrument Model 514). The probe was oriented at right angles to the electric field vector in air to minimize artifacts. Using the value of 0.88 cal/g °C for the specific heat of brain tissue [Cooper and Trezek, 1972], SAR was determined from $\Delta T/t$ values recorded at the localized point in the brain of a dead rat at two levels of incident power.

At the localized point in the brain the values of $\Delta T/t$ were 0.010 and 0.017 °C/s at 60 mW/cm², and 100 mW/cm², respectively, providing estimates of the local SAR of 0.613 and 0.626 mW/g/mW/cm². These SAR values, determined at a point about 2 mm beneath the irradiated surface, are understandably greater than the SAR averaged over the entire irradiated volume. The average SAR was estimated to be within the 0.18–0.42 mW/g/mW/cm² range.

Temperature Regulation

Each animal was maintained at the selected temperature for at least 5 min to establish an NADH fluorescence baseline prior to sham or microwave exposure. In the hyperthermia plus microwave exposure experiments each rat had its brain temperature regulated to within -0.1 to $+0.05$ °C of 39 °C for a minimum of 5 min prior to microwave exposures (591 MHz, CW, 13.8 mW/cm²) of 0.5, 1.0, 3.0, and 5 min. Based upon an average SAR of 0.18–0.42 mW/g/mW/cm², the 13.8-mW/cm² exposure would result in an SAR for brain to be within 2.48–5.80 mW/g.

Biochemical Procedures

Immediately after completion of the sham or microwave exposure, the head, neck, and upper thorax were rapidly immersed in liquid nitrogen. After 2 min, the

frozen head was decapitated and stored in liquid nitrogen until the ATP and CP assay. The brain was removed from the skull and pulverized in an ethanol-dry ice-cooled (-35°C) tissue pulverizer; the frozen powder thoroughly mixed, and an aliquot of 0.15–0.25 g placed on top of 1.5 ml perchloric acid in a previously cooled (liquid N_2) test tube and weighed. The test tube was placed in a bath at -10°C for 25 min with gentle swirling of each test tube at 5-min intervals. After the 25 min all samples were analyzed for [ATP] and [CP] by the method of Lowry and Passoueau (1972).

Earlier studies had shown brain [ATP] decreasing almost simultaneously with decreasing [CP]. A hypoxia experiment was performed to show the normal energy relationship between [ATP] and [CP] where hypoxia was the only exogenous stress. Another group of animals with brain temperature of 35.6°C were subjected to 2% O_2 plus 98% N_2 for 0.5, 1, and 2 min. ATP and CP assays were performed on the brain of each animal as detailed above.

RESULTS AND DISCUSSION

The brains from the first 10 urethane-anesthetized control animals (sham exposure), with brain temperature of 35.6°C gave brain [ATP] and [CP] of 2.47 ± 0.03 and $3.44 \pm 0.11 \mu\text{mol/g}$ (mean $\pm$ SEM), respectively. These were not significantly different from previously reported values [Sanders et al, 1980]. Each subsequent set of sham or microwave exposures at 37.0 or 39.0 or 41.0°C had a group of 35.6°C controls. Thus, the total number of sham-exposed (35.6°C) controls was 51. Figure 2 shows the effect of temperature only on brain [ATP] and [CP]. Based on these data, 39.0°C was selected as representative of an increased temperature effect (hyperthermia) for comparison of hyperthermia plus microwave exposure and normothermic (35.6°C) sham and microwave exposure.

The work previously reported [Sanders et al, 1980] used sodium pentobarbital as an anesthetic agent. Urethane was selected for these studies because it gives longer periods of deep anesthesia and avoids barbiturate effects on energy metabolism, it was necessary to ascertain if there were significant differences in brain [ATP] and [CP] in sham or microwave exposures due to the anesthesia. Groups (six rats/group) of urethane-anesthetized animals with brain temperature maintained at 35.6°C were exposed to 591-MHz, CW radiation at 13.8 mW/cm^2 for 0, 0.5, 1, 3, and 5 min. Brain ATP and CP were assayed, as described above. The maximum deviation of any urethane-anesthetized brain [ATP] and [CP] for any group was $\leq 4.5\%$ when compared with the pentobarbital-anesthetized groups at the corresponding exposure interval. The 35.6°C brain [ATP] and [CP] data include the urethane and sodium pentobarbital-anesthetized, sham-exposed brain [ATP] and [CP] data.

The measurement of brain NADH fluorescence is complicated by the wide variability in the network of blood vessels immediately above and on the surface of the cerebral cortex. Since such variation resulted in large differences in the location of the excitation focal spot, voltage on the photomultiplier tube, and amplifier gain, it was not considered valid to group all data for statistical analyses. Each animal served as its own control. The NADH fluorescence levels observed during microwave exposure were compared with the preexposure NADH fluorescence level. Consequently, Figure 3 shows brain-NADH-fluorescence curves of single animals that are representative of the curves obtained from a minimum of six animals during micro-

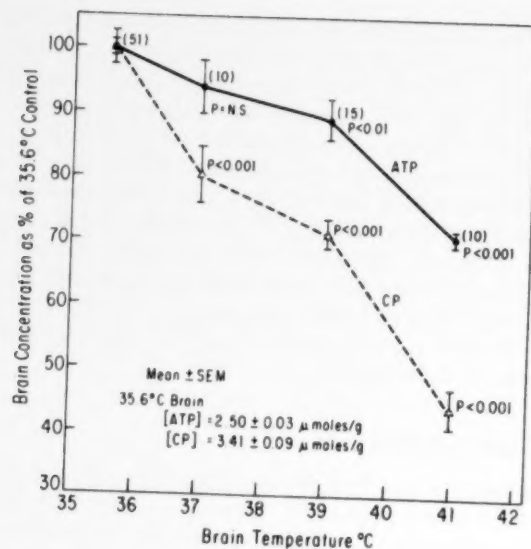


Fig. 2. Brain [ATP] and [CP] versus brain temperature.

wave exposures. The control baseline fluorescence was maintained for a minimum of 5 min prior to microwave exposure. This corresponds to the zero point on the % NADH fluorescence increase versus exposure time curves. The postexposure data are not shown since, in general, the brain NADH fluorescence returned to the baseline control level within 1 min.

The results of increasing the temperature of rat brain on [ATP] and [CP] are shown in Figure 2. The decreases in [ATP] and [CP] observed with the increase in temperature (37, 39, and 41 °C) were significantly different (Student's t-test for a two-group comparison, $P < .01$) from the control values (35.6 °C), with the exception of the [ATP] in the 37-°C brain.

Figure 3 shows the results of the microwave exposures on brain [ATP], [CP], and NADH fluorescence for the 35.6-°C brain, the 39-°C brain, and the effects of hypoxia on the 35.6-°C animals. Note that in the hypoxic animals brain [ATP] did not decrease until [CP] decreased below 59% of control levels. This illustrates the functional role of CP as a support system for maintaining [ATP] within narrow limits. Brain [CP] will decrease to levels of approximately 55–60% of control levels at normothermic conditions *before* any significant decrease in [ATP] can be observed.

In the microwave exposures of the 35.6 °C brain, [ATP] decreased to 74.8% of control at 0.5 min even though the [CP] had only decreased to 60.4%. The observed decreases in brain [ATP], when [CP] was at a level that would sustain normal [ATP], is consistent with the concept of a direct microwave inhibition of mitochondrial ATP production (oxidative phosphorylation). Previous work [Sanders et al, 1980] showed that with bilateral skull apertures the brain temperature decreased 0.1 °C during a 5-

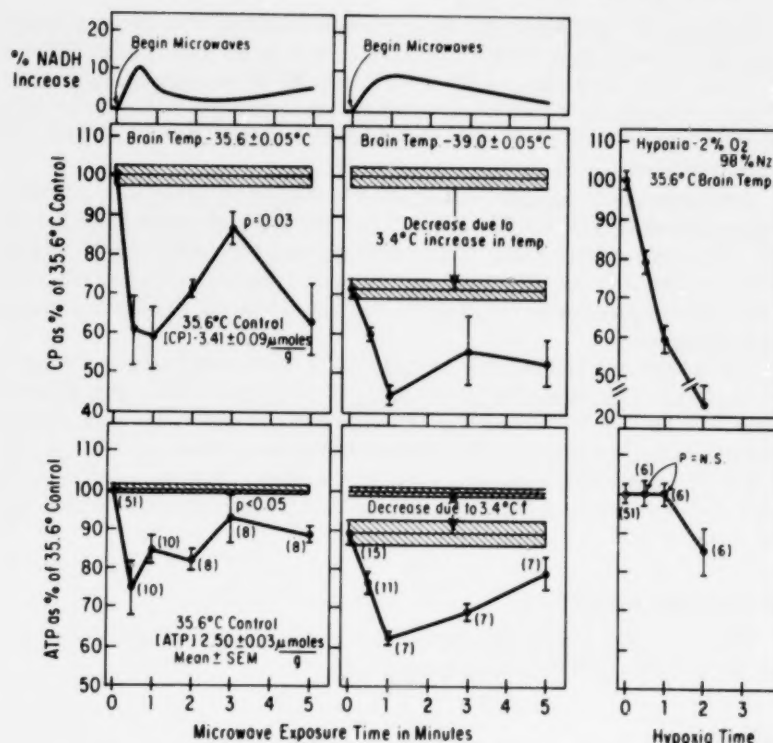


Fig. 3. Rat brain NADH fluorescence and [ATP] and [CP] changes at 35.6 and 39 °C with and without microwave exposure at 591 MHz, CW, 13.8 mW/cm²; and rat brain [ATP] and [CP] in hypoxia at 35.6 °C. Unless shown, $P < .01$ by unpaired Student's *t*-test between groups. NS = not significant.

min, 591 MHz, 13.8 mW/gm² microwave exposure. Thus, the observed decreases in brain [ATP] and [CP] could not be attributed to a general increase in the temperature of the brain.

The brain NADH fluorescence response to microwave exposure shows a rapid increase in NADH fluorescence immediately after microwave exposure begins. It could be argued that the decrease in [ATP] is due to increased ATP utilization by extramitochondrial cell functions. When ATP utilization increases, ADP is rapidly returned to the mitochondria and is available in greater concentrations. This results in an increased rate of mitochondrial electron transport chain activity. Therefore, the hydrogen atoms from the NADH molecules are transferred more rapidly down the chain. This results in a shift in kinetics of the NADH NAD redox state toward NAD—the oxidized state—which results in a decrease in NADH concentration [NADH]. The NADH fluorescence level increased immediately with the onset of microwave exposure. The data are consistent with the hypothesis of direct microwave inhibition of mitochondrial electron transport chain function of ATP production.

Results at 39 °C indicate that the increase in metabolic rate due to temperature affected the relationship between [CP] and [ATP] in brain. The [CP]/[ATP] ratio at 35.6 °C was 1.36 compared to 1.09 at 39 °C. At 39 °C the increase in demand for ATP due to the higher, heat-induced metabolic rate could not be met by an increase in the mitochondrial ATP-production rate and/or increase in CP-kinase activity. Thus, new levels of brain [ATP] and [CP] are observed.

The temperature regulator was adjusted so that power would turn off when the selected temperature (35.6 or 37 or 39 °C) was reached. The observations during the 5-min microwave exposure that (1) temperature never went above 39.05 °C and (2) the heating pads were turned on at least once during microwave exposure, indicate that the resultant decrease in [ATP] and [CP] could not be due to microwave-induced brain hyperthermia.

The decreases in [ATP] and [CP] in the 39 °C brain during microwave exposure were significant and resulted in [ATP] and [CP] being much lower than observed at the 35.6 °C. Thus, at 39 °C when the brain metabolic rate was increased, subsequent microwave exposure rapidly induced further decreases in [ATP] and [CP], similar to the 35.6 °C microwave exposure data, ie, without a further increase in brain temperature, are consistent with the concept of direct microwave inhibition of energy metabolism.

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